

DISTRIBUTION AND MORPHOLOGICAL CHARACTERISTICS OF *ARCEUTHOBIUM HONDURENSE* AND *A. NIGRUM* (VISCACEAE) IN MEXICO

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ABSTRACT

The geographic and host distributions of *Arceuthobium hondurense* and *A. nigrum* (Viscaceae) in southern Mexico and Central America have remained unclear due to difficulties in their identification. This study was conducted to clarify the morphology, phenology, distribution, and host affinities of these dwarf mistletoes. Morphological measurements were made for these species across their geographic distributions and nr ITS sequences were generated and compared from selected populations in Mexico. Although subtle, interspecific differences in plant morphology were found, *A. hondurense* and *A. nigrum* can be differentiated by the dimensions of their staminate spikes, staminate flowers, and fruits. Our data also indicated that *A. hondurense* and *A. nigrum* were not sympatric. *Arceuthobium hondurense* is distributed from northern Nicaragua to northern Oaxaca, Mexico and *A. nigrum* is distributed from Veracruz to northern Durango. Although *A. hondurense* and *A. nigrum* flowered in the fall, *A. hondurense* consistently peaked in September and *A. nigrum* peaked in October, extending into January in central Mexico. A spring flowering period reported previously for *A. nigrum* was not observed. Additional information on the host distribution and molecular differences of these dwarf mistletoes is also presented.

KEY WORDS: *Arceuthobium hondurense*, *Arceuthobium nigrum*, dwarf mistletoe, geography, hosts, ITS, molecular identification, parasitic plants

RESUMEN

La distribución geográfica de *Arceuthobium hondurense* y *A. nigrum* (Viscaceae) y de sus hospedadores en México y Centro América son inciertas debido a los problemas en su identificación. En este trabajo, se estudió la morfología, fenología, distribución y afinidades de hospedadores de estas dos especies de muérdagos enanos. Las mediciones morfológicas fueron hechas de poblaciones Mexicanas para ambas especies y se generaron y compararon secuencias de nr ITS. Aunque ligeras, existen diferencias interespecíficas en la morfología de las plantas, *A. hondurense* y *A. nigrum* se pueden distinguir por las dimensiones de las espigas estaminadas, flores y frutos. Nuestros datos indican que estas especies no se solapan en su distribución geográfica. *Arceuthobium hondurense* se distribuye desde el norte de Nicaragua hasta el norte de Oaxaca, México, mientras que *A. nigrum* se distribuye desde Veracruz hasta el norte de Durango, México. Aunque las dos especies florecen en el otoño, *A. hondurense* tiene su pico de floración en Septiembre y *A. nigrum* tiene su pico de floración en Octubre, extendiéndose hasta Enero en el Centro de México. El periodo de floración que fue reportado previamente para *A. nigrum* en la primavera no fue observado para esta especie. Se presenta información adicional sobre los hospedadores y sobre las diferencias moleculares entre estas especies de muérdago enano.

PALABRAS CLAVE: *Arceuthobium hondurense*, *Arceuthobium nigrum*, dwarf mistletoe, geography, hosts, ITS, molecular identification, parasitic plants

The genus *Arceuthobium* (Santalales: Viscaceae) consists of 42 species (Hawksworth & Wiens 1996) that are aerial parasites of Pinaceae or Cupressaceae. Many of the species, commonly known as dwarf mistletoes, are recognized as serious forest pathogens (Hawksworth & Wiens 1996; Mathiasen et al. 2008). Morphological characters consistent throughout the genus include small flowers produced on male and female plants, leaves reduced to squamate scales, and morphologically similar bi-colored fruits (Hawksworth & Wiens 1996). The genus has long been considered a taxonomically difficult group because of the extreme morphological reduction associated with the parasitic habit and the morphological similarities between species (Hawksworth & Wiens 1996). Factors that complicate classification and identification include a large amount of variation in morphology and geographic distributions as well as flowering periods that occasionally overlap. Two species that exemplify the problems associated with field identification of morphologically similar dwarf mistletoes occur in southern and central Mexico: *Arceuthobium hondurense* Hawksw. & Wiens (Honduran dwarf mistletoe) and *A. nigrum* Hawksw. & Wiens (black dwarf mistletoe).

Arceuthobium hondurense was originally described from central Honduras (Hawksworth & Wiens 1970) and thought to be extremely rare, even on the verge of extinction due to rapid and extensive harvesting of its pine hosts (Hawksworth & Wiens 1972). However, Honduran dwarf mistletoe was later found to occur from northern Nicaragua, through much of Honduras into Chiapas, Mexico and as far north as central Oaxaca (Mathiasen et al. 2001; Mathiasen et al. 2002a; Mathiasen et al. 2003; Mathiasen et al. 2006; Mathiasen & Melgar 2006). Although it likely occurs in Guatemala, *A. hondurense* has never been confirmed there (Hawksworth & Wiens 1977; Mathiasen et al. 2003). Another dwarf mistletoe, originally described as *A. hawksworthii* Wiens and C.G. Shaw (Hawksworth's dwarf mistletoe), was recombined as a subspecies of *A. hondurense* (Mathiasen 2007). Therefore, *A. hondurense* presently consists of two subspecies: *A. hondurense* subsp. *hondurense* which occurs from Nicaragua north to Oaxaca, Mexico (Mathiasen et al. 2010) and *A. hondurense* subsp. *hawksworthii* (Wiens & C.G. Shaw) Mathiasen which is primarily distributed in the Mountain Pine Ridge area of Belize (Mathiasen 2007), but also has been reported from central Honduras (Mathiasen et al. 2002b).

Because plants of *Arceuthobium hondurense* subsp. *hondurense* (hereafter referred to as *A. hondurense*) are similar in size and color to those of *A. nigrum*, determining the geographic distribution of these species in southern Mexico has been difficult (Hawksworth & Wiens 1989, 1996; Mathiasen et al. 2001, 2002a, 2003, 2010). Although *A. nigrum* was thought to be distributed from northern Durango, Mexico into southern Mexico (Hawksworth & Wiens 1996), it is now thought to be distributed only as far south as central Mexico (Mathiasen et al. 2010). Further, both *A. hondurense* and *A. nigrum* produce red flowers that bloom in the fall. We have collected additional morphological data for *A. hondurense* and *A. nigrum* since 1998. Here we report our findings and discuss the distribution of these dwarf mistletoes in Mexico based on our field observations and morphological measurements. Because prior studies (Mathiasen et al. 2003; Nickrent et al. 2004) have successfully used ribosomal DNA (rDNA) sequence information to discriminate between *A. hondurense* and *A. nigrum*, we conducted additional analyses of the internal transcribed spacer (ITS) region for several populations of both species, particularly populations in central Mexico where these species may be sympatric (Nickrent et al. 2004). In addition, because *A. vaginatum* (Willd.) Presl subsp. *vaginatum* (Mexican dwarf mistletoe) is also morphologically similar to *A. hondurense* as well as *A. nigrum*, and often confused with them, we have provided information on how to distinguish these species from *A. vaginatum*. The primary objective of this study, however, was to provide additional data on how to discriminate *A. hondurense* from *A. nigrum*—and vice versa—and in so doing, better determine their geographic and host ranges. Morphological data and ITS sequences for *A. vaginatum* were taken from Hawksworth and Wiens (1996) and obtained from GenBank, respectively.

MATERIALS AND METHODS

Morphology and Phenology

To compare morphological characters we sampled 16 populations of *Arceuthobium hondurense* (two from Oaxaca, Mexico, one from Nicaragua, and 13 from previous work by Mathiasen (2007)) and 14 populations of *A. nigrum* from throughout its geographic range (Fig. 1). Plants were measured from the type locality for both mistletoe species (Hawksworth & Wiens 1965, 1970, 1977) (Fig. 1; locations 6 and 23). From each population, 10–20 male and 10–20 female plants were collected and the dominant shoot from each infection was used for morphological measurements. Characters measured were those used by Hawksworth and Wiens (1996) for taxonomic classification of *Arceuthobium*: height, basal diameter, third internode length and width, and color of male and female plants; mature fruit length, width, and color; seed length, width and color; length and width of staminate spikes; staminate flower diameters for 3- and 4-merous flowers; length and width of staminate flower petals; and, anther diameter and anther distance from the petal tip. Plants were measured within 24 hours after collection using a digital caliper and a Bausch and Lomb 7X hand lens equipped with a micrometer. Staminate spike and flower measurements were made during the peak of anthesis and fruit and seed measurements were made during the peak of seed dispersal. One-way analysis of variance (ANOVA) was used to examine the variance in the above characters for *A. hondurense* and *A. nigrum* and significant differences between means were determined using a Tukey's honestly significant difference (HSD) post-hoc test ($\alpha = 0.05$). All statistical analyses were performed using JMP 8.0.2 software (SAS Institute, Cary, NC).



FIG. 1. Approximate locations of populations sampled for *Arceuthobium hondurense* subsp. *hondurense* (open circles) in Honduras and Mexico and *A. nigrum* (dark circles) in Mexico. Populations that are not numbered are locations reported in Hawksworth and Wiens (1996). Plant material from locations in bold were used to obtain nuclear ribosomal DNA internal transcribed spacer sequences for *A. hondurense* and *A. nigrum* (Fig. 2). *Arceuthobium hondurense*: HONDURAS. Department Cortes: 1 – Cusuco National Park; Department Lempira: 2 – Celaque National Park; Department Francisco Morazan: 3 – Lepaterique [RLM0136]; 4 – La Estancia; 5 – Tatumbla; 6 – 22 km SE of Tegucigalpa on Pan American Hwy [RLM98107]; 7 – 2 km S of Valle de Angeles; 8 – 7.5 km S of Valle de Angeles; Department El Paraiso: 9 – San Lucas. NICARAGUA. Nueva Segovia. 10 – Mozonte. MEXICO. 11 – Chiapas: San Cristobal de las Casas; 12 – Oxchuc; Oaxaca: 13 – 32 km E of Ixtlan [RLM0994]; 14 – 15 km E of Oaxaca City [RLM0993]; 15 – Suchixtepec [RLM0998]; 16 – 21 km NE of Teotitlan [RLM1086].—*Arceuthobium nigrum*: MEXICO. Veracruz: 17 – Cofre de Perote [RLM0764]; 18 – 3 km S of Sierra de Agua [RLM1082]; Puebla: 19 – Los Hermanos; 20 – Tetla de Ocampo; Hidalgo: 21 – Los Durazos; 22 – Metepec; Durango: 23 – 50 km E of El Salto [RLM0778]; 24 – 11 km E of El Salto [RLM0779]; 25 – 3 km E of El Salto [RLM1105]; 26 – 23 km N of Route 40 on road to San Miguel de Cruces [RLM0781]; 27 – 102 km N of Route 40 on road to San Miguel de Cruces; 28 – Otinapa; 29 – 30 km W of Santiago Papasquiaro; 30 – 18 km W of Tepehuanes.

Because the times of flowering and seed dispersal for *Arceuthobium hondurense* and *A. nigrum* are poorly known (Hawksworth & Wiens 1996), additional observations of the phenology of these taxa were made during the spring and fall of 1999, 2003, 2005, 2007, 2008, and 2010 as well as during the early spring of 2011.

DNA Extraction and ITS Sequencing

Samples of DNA were obtained from five and six specimens, each representing a geographically separate population, of *Arceuthobium hondurense* and *A. nigrum*, respectively. Locality and voucher number for each specimen (bold print) are presented in Fig. 1. For each specimen, total DNA was extracted using the DNeasy™ Plant Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. DNA purity and concentration were quantified for each sample using a NanoDrop ND-1000 (Thermo Fischer Scientific, Wilmington, DE). Full-length, ITS sequences (comprising ITS1, 5.8S rDNA gene, and ITS2) were PCR-amplified using the primer pair 18S 1830for and 26S 40rev (Nickrent et al. 2004). PCR amplifications were carried out in 25 μ L reaction mixtures containing 12.5 μ L of 2X AmpliTaq Gold® Master Mix (Applied Biosystems, Foster City, CA), 0.5 μ L of each 20 μ M primer, 11.25 μ L nuclease-free water, and ~2–18 ng (0.25 μ L of 8–78 ng/ μ L) of genomic DNA.

PCRs were performed in an Eppendorf Mastercycler[®] pro thermal cycler (Eppendorf, Westbury, NY) with the following cycling parameters: initial hold for 6 min. at 95°C; 5 cycles at 94°C for 30s, 55°C for 30s, and 72°C for 1 min.; 33 cycles at 94°C for 30s, 48°C for 30s, and 72°C for 1 min.; and, a final extension step of 72°C for 10 min. Blank reactions (i.e., minus genomic DNA) were run concomitantly to check for contamination of the reagents.

The size of each PCR product (bp) was checked separately by ultraviolet fluorescence after 1.2% agarose gel electrophoresis in 0.5x TAE buffer and staining with GelRed[™] (Phenix Research Products, Candler, NC). Amplification products were purified directly from reactions using ExoSAP-IT (0.4 µL per µL of reaction product; USB Inc., Cleveland, OH) and normalized to 130 ng per sequencing reaction. Sequencing was carried out using a BigDye terminators DNA sequencing kit (Applied Biosystems), ABI 3730 DNA sequencer, and the above forward and reverse primers. PCR products were sequenced in both directions. Sequences were proof-read and assembled in CodonCode Aligner (CodonCode Corporation, Dedham, MA). Boundaries to the 5'- and 3'-region of ITS1 and ITS2, respectively, were previously identified by Nickrent et al. (1994). ITS sequences for *A. hondurensis* (n=5) and *A. nigrum* (n=6) produced in this study were deposited in GenBank.

Phylogenetic Analysis

ITS sequences for *Arceuthobium hondurensis* and *A. nigrum* obtained in this study and from GenBank (*A. hondurensis* AY2888263 and *A. nigrum* AY288271) as well as *A. vaginatum* subsp. *vaginatum* (AY288286 and AY288287) and *A. douglasii* Engelman (L25687; outgroup) were included in the dataset. Sequences were aligned using ClustalX ver. 2 (Larkin et al. 2007) and visually edited as necessary in CodonCodon Aligner. Maximum Likelihood (ML) trees were constructed using PAUP* 4.0b10 (Swofford 2003). The DNA substitution model TIM2 and the parameter estimates for tree reconstruction were determined using the Akaike Information Criterion (AIC; Akaike 1974) as implemented in jModelTest 0.1.1 (Posada 2008). All nucleotides were included in the phylogenetic analysis; gaps were treated as missing characters. Heuristic searches were performed with 200 replicates of random sequence addition and tree bisection-reconnection (TBR) branch swapping. Branch support was evaluated using 1000 bootstrap replicates and 10 random additions of sequences per pseudo-replicate. Inter- and intraspecific genetic distances were also examined using Kimura's two-parameter model (K2P; Kimura 1980) for base substitution as implemented in PAUP*.

Bayesian analysis was also performed using MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). The best-fit model for DNA substitution was determined as described previously; however, Hasegawa-Kishino-Yano (HKY; Hasegawa et al. 1985) model and parameter estimates were determined using the Bayesian Information Criterion (BIC; Schwarz 1978). One cold and three heated Markov chain(s) were run, and samples were taken every 100 generations over 5.0×10^6 generations. The potential scale reduction factor (PSRF) for each of the model parameters was > 1.0 when the program was terminated. Stationarity was assessed by examining the average standard deviations of split frequencies and likelihood values. Burn-in value (10%) was determined using Tracer v1.5 (Rambaut & Drummond 2009). The remaining trees were used to calculate a 50% majority rule consensus tree and to determine the posterior probabilities.

RESULTS AND DISCUSSION

Arceuthobium hondurensis

Our measurements of *Arceuthobium hondurensis* indicate it forms larger plants than previously reported by Hawksworth and Wiens (1970, 1996); they reported plant heights averaged approximately 14 cm with a maximum height of 21 cm, but we measured plants (male and female combined) that averaged 22 cm. We found some male plants in Chiapas, Mexico that were over 65 cm in height (Table 1). The discrepancy in maximum heights is probably related to Hawksworth and Wiens only measuring specimens from central Honduras where plants were generally smaller than in southern Mexico (Mathiasen et al. 1999; Mathiasen 2007). The mean basal diameter of dominant shoots was the same as reported by Hawksworth and Wiens (1996); approximately 5 mm. However, we measured some shoots with basal diameters of nearly 13 mm, while Hawksworth and Wiens (1996) only reported a maximum of 9 mm for this character. The means and ranges for most of the remaining morphological characters were similar to those previously reported (Hawksworth & Wiens

TABLE 1. Morphological measurements for *Arceuthobium hondurense* and *A. nigrum*. Data are listed as mean (range) [n]. Means followed by different capital letters in the same row were significantly different using a Tukey's HSD post-hoc test ($\alpha = 0.05$). Lower case letters in brackets indicate sample sizes already listed in the same column. Plant heights in cm and all other measurements in mm.

| Character | <i>A. hondurense</i> | <i>A. nigrum</i> |
|----------------------------------|----------------------------|----------------------------|
| Plant Height | | |
| Male | 24.9 A (11.5–66.4) [160 a] | 24.3 A (10.3–53.5) [130 a] |
| Female | 18.6 A (9.1–33.1) [a] | 19.6 A (9.3–37.2) [a] |
| Basal Diameter | | |
| Male | 5.1 A (2.8–13.4) [a] | 7.0 B (4.4–12.5) [a] |
| Female | 5.4 A (2.8–12.8) [a] | 7.8 B (4.1–13.1) [a] |
| Length of Third Internode | | |
| Male | 16.1 A (6.6–34.0) [a] | 16.8 A (11.6–28.7) [a] |
| Female | 13.8 A (6.3–32.0) [a] | 16.5 B (11.8–31.8) [a] |
| Width of Third Internode | | |
| Male | 3.8 A (2.0–8.0) [a] | 4.9 B (4.0–7.8) [a] |
| Female | 4.0 A (2.0–10.0) [a] | 5.5 B (4.4–9.6) [a] |
| Staminate Spike Length | 14.3 A (6.1–17.9) [120 b] | 20.6 B (8.1–33.3) [200 b] |
| Staminate Spike Width | 1.7 A (1.3–2.4) [b] | 2.9 B (2.4–3.3) [b] |
| Mean Flower Diameter | | |
| 3-merous | 2.5 A (2.0–3.0) [80] | 3.2 B (2.7–4.0) [100 c] |
| 4-merous | 3.1 A (2.8–3.4) [40] | 4.8 B (3.6–5.4) [c] |
| Petal Length | 1.3 A (1.0–1.6) [b] | 1.7 B (1.3–2.3) [b] |
| Petal Width | 1.2 A (0.8–1.4) [b] | 1.4 B (0.8–1.9) [b] |
| Anther Diameter | 0.5 A (0.4–0.6) [b] | 0.8 B (0.5–1.1) [b] |
| Anther Distance from Tip | 0.3 A (0.2–0.6) [b] | 0.5 B (0.3–0.6) [b] |
| Fruit Length | 5.3 A (5.0–6.0) [100 c] | 6.9 B (5.2–8.8) [c] |
| Fruit Width | 3.4 A (2.8–4.2) [c] | 4.1 B (3.4–5.0) [c] |
| Seed Length | 3.1 A (2.8–3.5) [c] | 3.1 A (2.7–3.9) [c] |
| Seed Width | 1.5 A (1.3–1.7) [c] | 1.5 A (1.3–1.9) [c] |

1970, 1996) with two exceptions: the fruits and petals. The means for each of these characters were greater than that found by Hawksworth and Wiens (1996). The means for male and female plant heights, the third internode length of male plants, and seed length and width were not significantly different, but the means of the remaining characters we measured (e.g., width of the third internode and staminate spike length and width) were significantly different between *A. hondurense* and *A. nigrum* (Table 1). Our measurements of 4-merous flower diameters of *A. hondurense* ($n=40$) were the first reported for this character.

An important and consistent character exhibited by *Arceuthobium hondurense* was the dark red surface of the adaxial side of petals reported by Hawksworth and Wiens (1970, 1996), which was characteristic of this species throughout its geographic range. Another characteristic of *A. hondurense* that we observed, and reported by Hawksworth and Wiens (1970, 1996), was that the lower nodes of older plants were often swollen and round, this was particularly evident on old, male plants. Hawksworth and Wiens (1970) indicated that the stigmas on female flowers and fruits of *A. hondurense* were exerted as much as 0.5 mm. While we observed this character on many female plants, we also observed many flowers and fruits without exerted stigmas, suggesting that this characteristic was polymorphic for *A. hondurense*. Similarly, the nectary of *A. hondurense* was occasionally three-lobed as reported by Hawksworth and Wiens (1996), but again this characteristic was inconsistent.

Arceuthobium hondurense flowered from late August to November with its peak flowering period in mid- or late-September, dispersing seed at approximately the same time. This is consistent with what Hawksworth and Wiens (1996) reported, except we found that in some years it flowered well into November, which they did not observe.

Previously, Mathiasen et al. (2003) listed the pines parasitized by *Arceuthobium hondurense* in Central America and Chiapas, Mexico. Our observations support their findings, except that *A. hondurense* also parasitized *Pinus teocote* Schiede ex Schlechtendal & Chamisso in central Oaxaca. Although we have only observed *A. hondurense* infecting this pine in one location north of Ixtlan (Fig. 1, location 13), the level of infection (> 90%) indicated *Pinus teocote* was a principal host. This population was misidentified as *A. vaginatum* subsp. *vaginatum* by Hawksworth and Wiens (1996), as our morphological and ITS analyses indicated this population was indeed *A. hondurense*. Mistletoe plants on *P. teocote* at this location were dark brown to black, similar in color to *A. vaginatum*, but male plants flowered in the fall producing dark red flowers. Large male plants at this locality also had swollen nodes and parasitized *P. tecunumanii* Eguiluz et J. P. Perry, a principal host of *A. hondurense* elsewhere in southern Mexico (Mathiasen et al. 2003). In addition, *A. hondurense* was found parasitizing *P. lawsonii* Roehl ex Gordon & Glendinning in central and northern Oaxaca. Although Hawksworth and Wiens (1977, 1996) reported *A. hondurense* (but identified as *A. nigrum*) parasitizing *Pinus oaxacana* Mirov and *P. patula* Schiede ex Schlechtendal & Chamisso in Chiapas, we did not observe these host-mistletoe combinations during our field work in southern Mexico.

Arceuthobium nigrum

Arceuthobium nigrum is morphologically very similar to *A. hondurense*. Both species produced relatively large, dark brown to black plants on their pine hosts (Hawksworth & Wiens 1996). Male and female plants of *A. nigrum* averaged nearly 25 and 18 cm in height, respectively, but were not significantly larger than *A. hondurense* (Table 1). It is difficult to compare our results for plant heights with those of Hawksworth and Wiens (1996) because they only provided a range of heights for *A. nigrum* (15–35 cm, maximum = 45 cm). However, the largest plant we measured for *A. nigrum* was a male over 53 cm tall from Puebla, Mexico. We also found that the basal diameter of shoots averaged two-mm larger (7.6 mm) than that previously reported (5 mm, Hawksworth & Wiens 1996) with a maximum basal diameter nearly twice that described by Hawksworth and Wiens. Measurements of the third internode widths also indicated that *A. nigrum* produced thicker plants (about 5 mm) than what Hawksworth and Wiens reported (about 4 mm). The means for the basal diameters of male (7 mm) and female plants (7.8 mm) and third internode widths of *A. nigrum* were significantly greater than those for *A. hondurense* (Table 1). Furthermore, the mean length of the third internode of female plants of *A. nigrum* (16.5 mm) was significantly longer than those of *A. hondurense* (13.8 mm), but not the mean length of the third internode of male plants.

The only flower characteristics Hawksworth and Wiens reported for *Arceuthobium nigrum* was the diameter of 3-merous flowers (3.5 mm), which was slightly larger than the mean diameter for the 3-merous flowers we measured (3.2 mm). Our observations indicated that *A. nigrum* commonly produced 4-merous flowers also, thus, we sampled these flowers and found they averaged nearly 5 mm in diameter (Table 1). Collectively, the mean diameters of 3 and 4-merous flowers of *A. nigrum* were significantly larger than those of *A. hondurense*. Petal sizes were also relatively large for *A. nigrum* when compared to other dwarf mistletoes (Hawksworth & Wiens 1996). We found petals longer than 2 mm and nearly as wide, both significantly larger than those for *A. hondurense* (Table 1). Another key characteristic of *A. nigrum* flowers that was consistent throughout its geographic range, was that the adaxial surface of its petals was dark red. While this characteristic was easily observed, and is similar to flowers for *A. hondurense*, a review of the literature (Hawksworth & Wiens 1989, 1996) on *A. nigrum* revealed that the petal color, as a diagnostic character, had never been mentioned before. The reasons for this omission remain unclear.

Fruits of *Arceuthobium nigrum* were remarkably glaucous and large compared to other dwarf mistletoes. The mean fruit length was nearly 7 mm, which is what Hawksworth and Wiens reported. However, we found fruits 8.8 mm in length compared to 9.0 mm by Hawksworth and Wiens (1996). In contrast, the average width of fruits we examined was larger (4.1 mm) than that reported by Hawksworth and Wiens (3.5 mm). We measured seeds that were shorter, but wider on average than those examined by Hawksworth and Wiens (1996). The means for fruit length and width of *A. nigrum* were significantly larger than those of *A. hondurense*, however, the mean length and width of seeds were similar for both species (Table 1).

The phenology of *A. nigrum* requires additional observations as we were unable to confirm the incidence of two flowering periods—one in March–April and one in September–October—as reported previously by Hawksworth and Wiens (1989, 1996). We examined male plants of *A. nigrum* at several localities in mid- to late-March during 2003, 2005, and 2007 as well as in early April 2011 and never observed open flowers. Furthermore, staminate flowers did not appear to be approaching anthesis in late March or early April. Our field observations, however, indicated that it flowers beginning in mid-September and continued into November in Durango, Mexico, while in central Mexico, *A. nigrum* flowers in late-September and continued into at least January. Peak flowering was in early October in Durango; however, the peak flowering period in central Mexico is still poorly understood. Seed dispersal initiated in early September and peaked in mid-October continuing to mid-November in Durango and elsewhere in central Mexico.

Our field observations of pines infected by *Arceuthobium nigrum* in Mexico did not reveal any additional hosts. The principal hosts of *A. nigrum* in Durango were clearly *Pinus leiophylla* Schiede ex Schlechtendal & Chamisso, *P. lumholtzii* B. L. Robinson & Fernald, *P. teocote*, and *P. chihuahuana* Engelman as reported by Hawksworth and Wiens (1996). This mistletoe also been reported to rarely infect *P. arizonica* Engelman and *P. cooperi* Blanco in northern Mexico (Hawksworth & Wiens 1996), but we have not observed it on these infrequent hosts. In central Mexico its principal host was *P. teocote*. *Pinus patula* Schlechtendal & Chamisso was a secondary host at several locations in Hidalgo and Puebla. While we agree with the classification of *P. pseudostrobus* Lindley as an occasional host of *A. nigrum* (Hawksworth & Wiens 1996), we were unable to verify whether *P. montezumae* A. B. Lambert was also an occasional host. Moreover, we did not observe any *P. montezumae* at the location where Hawksworth and Wiens reported an infestation of *A. nigrum* in Hidalgo nor in Veracruz where we found large, mistletoe-free *P. montezumae* growing near *P. teocote* severely-infected with *A. nigrum*. Therefore, the susceptibility of *P. montezumae* to *A. nigrum* needs further study. Although Hawksworth and Wiens (1989, 1996) reported that both *P. lawsonii* and *P. oaxacana* Mirov were principal hosts of *A. nigrum*, this host susceptibility classification was based on infection of these pines by *A. hondurense* in Oaxaca and Chiapas, respectively (Mathiasen et al. 2003).

DNA Analyses

DNA sequence analysis demonstrated that *Arceuthobium hondurense*, *A. nigrum*, and *A. vaginatum* occurred in three well supported clades (Fig. 2). All samples identified morphologically as *A. hondurense* and *A. nigrum* yielded a 627 and 623 bp fragment, respectively, consisting of the 3' end of the 18S (4 bp), complete ITS1-5.8S-ITS2 sequence (604 and 600 bp), and the 5' end of the 26S (19 bp). Four of five sequences for *A. hondurense* were identical (mean K2P value = 0.0017); however, RLM 98107 differed by an A/G nucleotide change at positions 22 and 40 in ITS1. Similarly, ITS sequences for *A. nigrum* were nearly identical (mean K2P value = 0.0018); except for A/T nucleotide changes at position 508 in ITS2. The alignment for phylogenetic analyses consisted of 649 characters including those of *A. douglasii* and *A. vaginatum*. Of these characters, 574 were constant, 50 were parsimony-informative, and 25 were parsimony-uninformative. The combined, ML and Bayesian consensus tree supported three distinct clades (Fig. 2) with bootstrap values $\geq 98\%$ and posterior probability values equal to 1.00, respectively. Each plant identified according to morphometric data as *A. hondurense* or *A. nigrum* formed a distinct clade with either *A. hondurense* (RLM 0136, Nickrent et al. 2004) or *A. nigrum* (DLN 2019, Nickrent et al. 2004). Sequences of *A. nigrum* differed from those of *A. hondurense* and *A. vaginatum* by approximately 43 nucleotides (mean nucleotide difference = 43.07, mean K2P value = 0.0775). Likewise, the mean number of nucleotide changes between *A. hondurense* and *A. vaginatum* was 13.0 (mean K2P value = 0.0224).

Nickrent et al. (2004) reported that *A. hondurense* may occur in Veracruz based on molecular data (GenBank accession no. L25693; voucher DLN 2018), but our results did not support this. Plants collected south of Sierra de Aqua (RLM 1083), the approximate location where Nickrent collected DLN 2018 (D. Nickrent, pers. comm.), were morphologically similar to those of *A. nigrum*. As suspected, the ITS sequences generated from RLM 1083 were identical to L25693 (data not shown). However, in a separate phylogenetic analysis (data not shown), these collections/sequences were unrelated to *A. nigrum* (AY288271) as well as GenBank accessions of *A. durangense* Hawksw. & Wiens, *A. gillii* Hawksw. & Wiens, *A. hondurense*, and *A. vaginatum*. Nickrent previously

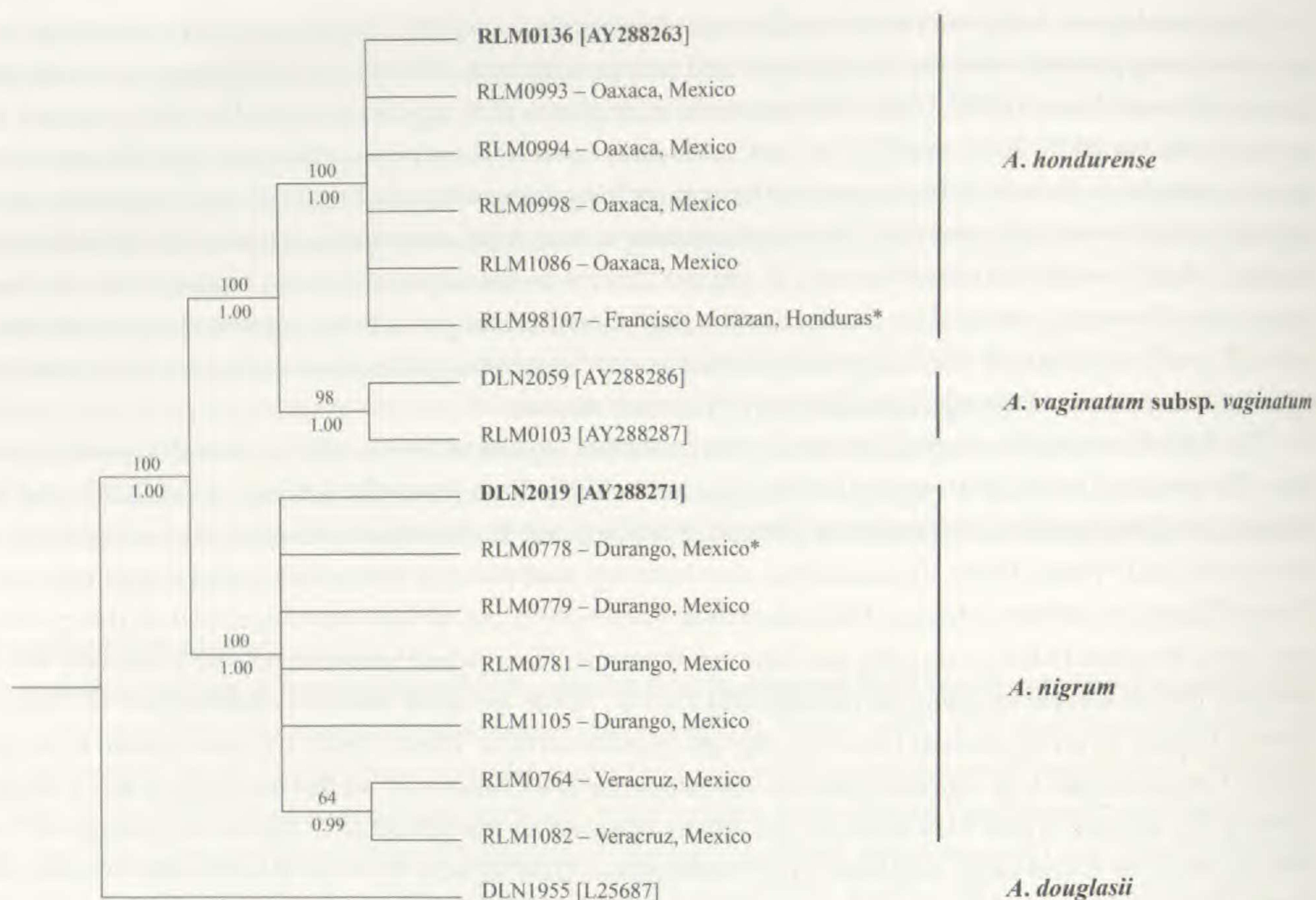


FIG. 2. Fifty-percent (50%) majority rule consensus tree based on maximum likelihood and Bayesian analyses using nuclear ITS sequences of *Arceuthobium nigrum*, *A. hondurensis*, *A. vaginatum* subsp. *vaginatum*, and the outgroup taxon, *A. douglasii*. Representative sequences of *A. nigrum* and *A. hondurensis* used in Nickrent et al. (2004) in bold. Sequences for *A. douglasii* and *A. vaginatum* ssp. *vaginatum* were obtained from GenBank ([] = accession number). Collector abbreviations are for Robert L. Mathiasen (RLM) and Daniel L. Nickrent (DLN) and precede assigned voucher numbers followed by locality (department/state – country; * = type locality). Numbers above branches indicate PAUP bootstrap values >60% (after 10^3 replicates), numbers below are Bayesian posterior probabilities >0.90 (after 5.0×10^6 generations).

TABLE 2. Principal morphological and physiological characteristics distinguishing *Arceuthobium hondurensis*, *A. nigrum*, and *A. vaginatum* subsp. *vaginatum*. All measurements in mm and ranges in parentheses. Data for *A. vaginatum* from Hawksworth and Wiens (1996).

| Character | <i>A. hondurensis</i> | <i>A. nigrum</i> | <i>A. vaginatum</i> |
|--------------------------------------------|-----------------------|------------------|---------------------|
| Mean Basal Diameter ^a | 5.3 (2.8–12.8) | 7.4 (4.1–13.1) | 7.0 (4–20) |
| Mean Width of Third Internode ^a | 3.9 (2.0–8.0) | 5.2 (4.0–9.6) | 5.0 (2.8–8.5) |
| Swollen nodes at base of older plants | Yes | No | No |
| Staminate Spikes | | | |
| Secondary Branching | No | No | Yes |
| Mean Width | 1.7 (1.3–2.4) | 2.9 (2.4–3.3) | 2.0 (1.5–2.5) |
| Mean Flower Diameter | | | |
| 3-merous flowers | 2.5 (2.0–3.0) | 3.2 (2.7–4.0) | 3.5 ^b |
| 4-merous flowers | 3.1 (2.8–3.4) | 4.8 (3.6–5.4) | 4.5 ^b |
| Red flowers | Yes | Yes | No |
| Mean Petal Length | 1.3 (1.0–1.6) | 1.7 (1.3–2.3) | 1.6 ^b |
| Mean Petal Width | 1.2 (0.8–1.4) | 1.4 (0.8–1.9) | 1.1 ^b |
| Mean Fruit Length | 5.3 (5.2–6.0) | 6.9 (5.2–8.8) | 5.5 ^b |
| Mean Fruit Width | 3.4 (3.1–4.2) | 4.1 (3.4–5.0) | 3.5 ^b |
| Anthesis | Aug–Nov | Sep–Jan | Mar–Apr |
| Seed Dispersal | Aug–Sep | Sep–Oct | Aug |

^a – Male and female plants combined.

^b – No range provided in Hawksworth and Wiens (1996).

identified DLN 2018 (L25693) as either *A. vaginatum* (Nickrent et al. 1994) or *A. hondurense* (Nickrent et al. 2004). The species identity of this mistletoe (RLM 1083 and DLN 2018), therefore, remains unresolved and requires further study.

SUMMARY

Although *Arceuthobium hondurense*, *A. nigrum*, and *A. vaginatum* are morphologically similar and often difficult to distinguish from each other *in situ*, there are several diagnostic characteristics that can be used to identify them in central Mexico where they may be sympatric. Our results support the classification of these taxa as distinct species and the principal morphological and physiological characters that can be used to distinguish these species are summarized in Table 2. While the overall height of male and female plants and their color cannot be used to easily separate these species, *A. hondurense* is a more slender plant than both *A. nigrum* and *A. vaginatum*. It also has swollen, rounded nodes, particularly near the base of older plants. This characteristic is most evident on older, male plants. In contrast, *A. nigrum* and *A. vaginatum* lack swollen, rounded nodes near the base of plants.

Another key characteristic of *A. hondurense* that separates it from the other dwarf mistletoes is the width of its staminate spikes. While the length of staminate spikes often is too variable to be of any diagnostic value, the width of the staminate spikes of *A. hondurense* are thinner (mean 1.7 mm) compared to those of *A. nigrum* (2.9 mm) and *A. vaginatum* (2.0 mm). Furthermore, the staminate spikes of *A. nigrum* and *A. hondurense* generally do not form secondary branches, while those of *A. vaginatum* typically do.

Arceuthobium hondurense primarily forms 3-merous flowers, and occasionally 4-merous flowers, but *A. nigrum* and *A. vaginatum* commonly form both 3- and 4-merous flowers. Although the adaxial surface of petals of male flowers for *A. hondurense* and *A. nigrum* is distinctively dark red, the 3-merous flowers of *A. hondurense* are smaller (2.5 mm) on average than those of *A. nigrum* (3.5 mm). The color of male flower petals of *A. vaginatum*, however, is dark brown to green. While the fruits of both *A. hondurense* and *A. nigrum* are usually markedly glaucous, the fruits of *A. nigrum* are larger than those of *A. hondurense* as well as *A. vaginatum*. Additionally, *A. hondurense* and *A. nigrum* primarily flower from late August through September and October, but *A. vaginatum* flowers from March through April (Hawksworth & Wiens 1965, 1996). Additional observations of *A. nigrum* are still necessary to determine if it flowers in the spring as reported by Hawksworth and Wiens (1989, 1996). Our observations of *A. nigrum* over multiple seasons and years in Durango, Mexico, do not support a spring flowering period for *A. nigrum*. Furthermore, our analyses confirm that these species can be readily distinguished using ITS-rDNA sequences as previously demonstrated by Nickrent et al. (1994, 2004).

The host specificity of these mistletoes may help separate them, depending on the locality in Mexico. In Durango, *Arceuthobium nigrum* and *A. vaginatum* both parasitize *P. teocote*, but *P. teocote* is less susceptible to *A. vaginatum* (a secondary host) (Hawksworth & Wiens 1996). Moreover, *A. vaginatum* does not parasitize *P. leiophylla*, *P. lumholtzii*, nor *P. chihuahuana*, which are all highly susceptible to *A. nigrum*. In central Mexico, the principal host of *A. nigrum* is *P. teocote*, but *P. patula* is also infected by *A. vaginatum* there. Now that *A. hondurense* has been discovered severely infecting *P. teocote* in Oaxaca, Mexico, infection of this pine cannot be used to distinguish *A. nigrum* from *A. hondurense*, since these mistletoes both flower in the fall, have red flowers, and are similar in size and color. The width of internodes and staminate spikes, therefore, are likely the best characters for distinguishing between them. The size of 3-merous flowers, petals, and fruits will also assist in distinguishing *A. nigrum* (larger flowers and fruits) from *A. hondurense* (Table 2).

Based on our field observations and measurements of plant characteristics of the dwarf mistletoes in southern Mexico, we do not agree that *Arceuthobium nigrum* or *A. vaginatum* occur in Oaxaca or Chiapas. These species are primarily distributed along the Central Volcanic Cordillera of central Mexico and north into Durango. *Arceuthobium vaginatum* extends as far north as central Chihuahua in the Sierra Madre Occidental and as far north as southern Coahuila in the Sierra Madre Oriental (Hawksworth & Wiens 1996). However, the geographic distribution of *A. nigrum* is centered on the eastern side of the Central Cordillera and extends north into Durango (Fig. 1). *Arceuthobium vaginatum* is sympatric with *A. nigrum* in central Mexico (Hawksworth &

Wiens 1996) and since it also extends as far north as Chihuahua, it is probably sympatric with *A. nigrum* in Durango.

Honduran dwarf mistletoe, which was once thought to be near extinction (Hawksworth & Wiens 1972), is now known to be distributed from northern Nicaragua to northern Oaxaca, Mexico (Fig. 1). Our surveys in 2010 confirmed that *Arceuthobium hondurense* occurs in northern Oaxaca (Fig. 1, location 16), so we now know this species occurs almost to Veracruz and Puebla in central Mexico. Moreover, it has only been found in widely-scattered, small populations throughout its geographic range and therefore, should not be considered a common parasite of its pine hosts. It should also be noted that the reports of *A. nigrum* in Guatemala and El Salvador (Hawksworth & Wiens 1977, 1989, 1996) should be considered as reports of *A. hondurense*. In addition, since our results demonstrated that at least one of the populations of *A. vaginatum* from north-central Oaxaca was misidentified by Hawksworth and Wiens (1996) and is indeed *A. hondurense* (Fig. 1, location 13), we suspect the southern distribution of *A. vaginatum* only extends into Puebla and not Oaxaca. Further investigations, however, are warranted to assess whether *A. vaginatum* occurs in Oaxaca as several collections of this species have been made in the Sierra Juárez near Ixtlan. While all of these were classified as *A. vaginatum* by Hawksworth and Wiens (1996, page 370), we suspect these collections represent additional populations of *A. hondurense* in Oaxaca, but this needs to be confirmed.

ACKNOWLEDGMENTS

The field assistance provided by Carolyn Daugherty and earlier reviews of the manuscript by Carolyn Daugherty, Dan Nickrent, and Job Kuijt are greatly appreciated. We also appreciate the help of Gustavo Perez, Socorro Gonzalez-Elizondo, and Juan Tun Garrido with reviews and Spanish translation for the Resumen.

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